

Patent Claims

1. Method for the identification of 5-methylcytosine positions in genomic DNA is characterized by the fact that the following method steps are conducted:

- a) the genomic DNA of a cell, a cell line, a tissue or an individual is chemically treated in such a way that cytosine and 5-methylcytosine react differently and a different base pairing behavior of the two products results in the duplex,
- b) the same nucleic-acid segment is amplified by means of a polymerase reaction,
- c) the same nucleic-acid segment of at least one other cell, cell line, tissue or individual or any reference DNA is treated according to steps a) and b),
- d) heteroduplexes are formed from the at-least two amplified products of steps b) and c),
- e) a detectable label is introduced into the heteroduplex by means of a reaction, which is specific for non-complementary base pairs.

2. Method according to ~~claim 1~~, further characterized in that only positions are used and indicated in which the cytosine methylation is variable between different cells, cell lines, tissues or individuals, for identification of differences in cytosine methylation patterns between different cells, cell lines, tissues and individuals.

3. Method according to ~~claims 1 to 2~~, further characterized in that disulfite (bisulfite, pyrosulfite) is utilized as the reagent for selective conversion of

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cytosine to uracil, whereby 5-methylcytosine remains unchanged, in step a) according to claim 1.

4. Method according to one of claims 1 to 3, further characterized in that genomic DNA of several individuals, tissues, cell lines or cells is amplified jointly in step b) of claim 1.

5. Method according to one of claims 1 to 3, further characterized in that genomic DNA of several individuals, tissues, cell lines or cells is amplified separately and then treated jointly according to step e) of claim 1.

6. Method according to one of claims 1 to 5, further characterized in that by formation of heteroduplexes from the DNA of different individuals, tissues, cell lines or cells, erroneous base pairings are produced at the positions at which a 5-methylcytosine was localized in the genomic DNA.

7. Method according to claim 1, further characterized in that in step d), by formation of heteroduplexes with a completely methylated reference DNA, erroneous base pairings occur at the positions at which cytosine was found in the genomic DNA.

8. Method according to claim 1, further characterized in that in step d), by formation of heteroduplexes with a completely demethylated reference DNA,

erroneous base pairings occur at those positions at which 5-methylcytosine was found in the genomic DNA.

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9. Method according to one of claims 6 to 8, further characterized in that the erroneous base pairings by means of "chemical mismatch cleavage" (chemical modification at non-complementary positions) lead to a specific or sufficiently selective backbone cleavage at these positions.

10. Method according to one of claims 6 to 8, further characterized in that the DNA is cleaved enzymatically specifically or sufficiently selectively at the erroneous base pairings.

11. Method according to one of claims 1 to 10, further characterized in that DNA fragments are obtained in step e) according to claim 1, whose size infers the cleavage positions and thus the position of methylcytosines and/or the methylation positions that differ between different individuals, tissues, cell lines or cells.

12. Method according to claim 11, further characterized in that the analysis of size (molecular weight) of the DNA fragments is conducted by means of mass spectrometry.

13. Method according to claim 12, further characterized in that the fragments are analyzed by means of matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI).

14. Method according to claim 12, further characterized in that the fragments are analyzed by means of electrospray ionization mass spectrometry (ESI).

15. Method according to claims 13 or 14, further characterized in that the size of the fragments produced in step e) according to claim 1 is adapted to the performance capacity of the mass spectrometer.

16. Method according to claim 15, further characterized in that several PCRs of a gene segment are introduced and the primers are set stepwise newly each time so that the fragment size to be expected each time at least in one of these PCRs falls in the mass range detectable by means of mass spectrometry.

17. Method according to claim 16, further characterized in that one of the PCR primers is positioned newly stepwise by the maximally detectable mass range of the mass spectrometer, relative to the other primer.

18. Method according to one of claims 1 or 2, further characterized in that in step b) one primer of the PCR is provided with a chemical function, so that the PCR product can be immobilized on a surface.

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19. Method according to one of claims 1 or 2, further characterized in that the PCR product produced in step b) is transferred to different reaction vessels and the surfaces of the reaction vessels are chemically treated such that the PCR product can be bound thereon.

20. Method according to one of claims 1 or 2, further characterized in that PCR products of different individuals that are produced in step c) are transferred into different reaction vessels prepared according to claim 19.

21. Method according to one of claims 1 or 2, further characterized in that an enzyme which forms a complex with a non-complementary base pair is used for step e).

22. Method according to claim 21, further characterized in that this enzyme is MutS.

23. Method according to claim 21, further characterized in that the enzyme bears a label, by which a complex can be displayed.

24. Method according to claim 21, further characterized in that the label is a fluorescence label, a chemiluminescence label, a mass label or a photochemically cleavable mass label.

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25. Method according to one of claims 1 to 24, further characterized in that one compares an amplified DNA sample according to step c) in claim 1, which displays a difference relative to an amplified DNA sample in step b), in a second run of the method itself, [with] a [similar] DNA sample according to step b) in claim 1 and with all other DNA samples to be investigated.

26. Method according to one of claims 1 to 25, further characterized in that a preselection of gene segments to be investigated in detail by the mass spectrometer is conducted by means of fluorescence labeling or chemiluminescence labeling of the immobilized DNA strand, the lack of which, after conducting steps d) and e) of claim 1 and a washing step, indicates the presence of methylated cytosines in the investigated genomic DNA segment.

27. Method according to one of claims 1 to 25, further characterized in that a preselection is made of the gene segments to be investigated in detail by mass spectrometry by means of a more non-specific variant according to claims 20-23.

28. Kit for conducting a method according to claim 1, comprising DNA of at least two individuals, tissues, cell lines or cells that are as different as possible, along with reagents, in order to indicate the variable methylation positions.

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29. Kit for conducting the method according to claim 1, comprising completely methylated and/or demethylated DNA and reagents, which are necessary for the detection of methylated cytosines in any DNA sample.

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